Comparing the Microlab® 600 to Volumetric Glassware and Air Displacement Pipettes

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HAMILTON®

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Abstract

Analytical sample preparation is used in many industries such as forensics, environmental chemistry, mining, beer and wine fermentation and many others. Concentrated samples are diluted and analyzed via High Performance Liquid Chromatography, Gas Chromatography, Atomic Absorption, and/or Inductively Coupled Plasma Spectroscopy to name a few. Traditionally these samples are prepared using volumetric glassware, syringes and/or pipettes. These manual techniques are effective but leave room for improvement. The use of volumetric glassware is time consuming and often results in waste due to large preparation volumes. Traditional air displacement pipettes are prone to fluctuations in accuracy resulting from user-to-user variation, sample vapor pressure, viscosity and atmospheric pressure. Using a positive displacement automated pipetting device like the Microlab® 600 can reduce sample preparation time, reduce waste and improve consistency of results. For example a 1:50,000 dilution can be performed in a single step due to the wide range of syringes available from 10 µL to 50 mL. In this study we will show that the Microlab 600 saves time, money, limits waste production and maintains high accuracy and precision.

Introduction

Analytical sample preparation is critical in many different industries like forensics, mining, environmental chemistry, etc. These industries have high standards for sample preparation. The equipment used needs to be easy to use, cost effective, and highly accurate and precise. It should also eliminate user-to-user variability and yield results that are highly reproducible. Sensitive equipment is used to analyze the diluted samples such as a Gas Chromatograph or a High Performance Liquid Chromatograph so variation must be limited. The methods used must also meet high standards set by the EPA requirements or N.I.S.T. traceability.

The most common techniques used to prepare samples are the use of volumetric glassware, pipettes, syringes or a combination of these. Each of these techniques offer different advantages. Volumetric glassware is used because of the high accuracy and precision achieved. It is very easy to use, provides minimal user-to-user variation and has high reproducibility. Pipettes are used because they are easy to use and they eliminate cross-contamination between samples. Syringes are used because they have high accuracy and precision at volumes into the microliter range.

Every technique will have advantages as well as disadvantages. Volumetric glassware is easy to use, however this technique is extremely time consuming. Once the glassware is used it must be rinsed out and cleaned prior to the next use. Volume sizes are limited; often times large sample volumes are made simply because it is the smallest glassware available causing excess waste of chemicals and buffer. Pipettes are an effective choice if the user has good technique. Pipettes are affected by atmospheric pressure, high viscosity solutions and user-to-user variation.

The techniques listed above are common for analytical sample preparation. In this study the focus is to compare the Microlab 600 to volumetric glassware and pipettes, specifically analyzing the performance in relation to accuracy and precision, cost effectiveness, waste produced and ease of use.

Methods & Results

Validating Equipment

Prior to running the experiment the Microlab 600 and Hamilton Pipettes were calibrated to N.I.S.T. traceable standards to meet our manufacturing requirements for accuracy and precision.

All of the volumetric glassware was validated for Class A compliance according to Class A standards. To test for compliance, purified water from a Millipore® Advantage A10 instrument was dispensed from each volumetric vessel and the mass of the water sample was measured and recorded using a Sartorius CPA124S balance. Each volumetric vessel was verified to be compliant at 10, 50 and 100 percent of the vessel total volume. Each dispense volume was repeated 10 times. Throughout the validation process the temperature of the water was monitored. To calculate the actual volume dispensed, the recorded mass was divided by the density of water at the measured temperature; reference Table 1 for more details.

Table 1: Density of Water at Various Temperatures

°C	g/mL	°C	g/mL
17	0.998774	24	0.997296
18	0.998595	25	0.997044
19	0.998405	26	0.996783
20	0.998203	27	0.996512
21	0.997992	28	0.996232
22	0.997770	29	0.995944
23	0.997538	30	0.995646

Taken from CRC Handbook of Chemistry and Physics, 50th Edition, 1969, page F-4.

The individual dispense volumes were averaged. The percent accuracy was calculated using the following equation:

$$\text{Accuracy (\%)} = (\text{Average} - \text{Expected Volume}) / \text{Expected Volume} \times 100$$

The precision was calculated using the following equation:

$$STDEV = \frac{\sqrt{(V_1 - V_{avg})^2 + (V_2 - V_{avg})^2 + (V_3 - V_{avg})^2 \ldots}}{10}$$

$$CV\ (\%) = STDEV / V_{avg} \times 100$$

Sample Preparation

A stock solution of 60 mg/mL phenol (TCI America, P1610) was diluted in 100% acetonitrile (Sigma, 34851-4L). All subsequent dilutions were made from this stock solution.

The dilution series were prepared in replicates of five. Each sample series was monitored for the time it took to prepare. Each technique was tested one at a time. The dilutions were prepared according to Table 2.

Table 2: Dilution Preparation

	Microlab 600 (1 mL and 25 µL syringes)			Volumetric Glassware (10 mL flask, 10 mL buret, 250 µL syringe)			Air Displacement Pipettes (1 mL, 300 µL, 25 µL, 10 µL)		
	Sample Volume (µL)	Diluent Volume (µL)	Final Volume (µL)	Sample Volume (µL)	Diluent Volume (µL)	Final Volume (µL)	Sample Volume (µL)	Diluent Volume (µL)	Final Volume (µL)
1:1	500	500	1,000	5,000	5,000	10,000	500	500	1,000
1:4	200	800	1,000	2,000	8,000	10,000	200	800	1,000
1:9	100	900	1,000	1,000	9,000	10,000	100	900	1,000
1:49	20	980	1,000	200	9,800	10,000	20	980	1,000
1:99	10	990	1,000	100	9,900	10,000	10	990	1,000
1:199	5	995	1,000	50	9,950	10,000	5	995	1,000

HPLC Analysis

Once all the samples were prepared they were analyzed via an Agilent 1100 HPLC system with a Hamilton PRP™-1, 5 µM 2.1 x 150 mm column. Analytical conditions—flow rate: 0.25 mL/min; temperature: ambient; injection volume: 1 µL; mobile phase: 70:30 Acetonitrile:H_2O (isocratic); and detection: UV at 254 nm.

Results

The phenol peak areas for all five replicates of the dilution series were averaged and plotted for each technique in Figure 1. A best fit line and R^2 value was determined for each technique. All techniques showed a high correlation but the Microlab 600 was the highest with a value of 0.9992.

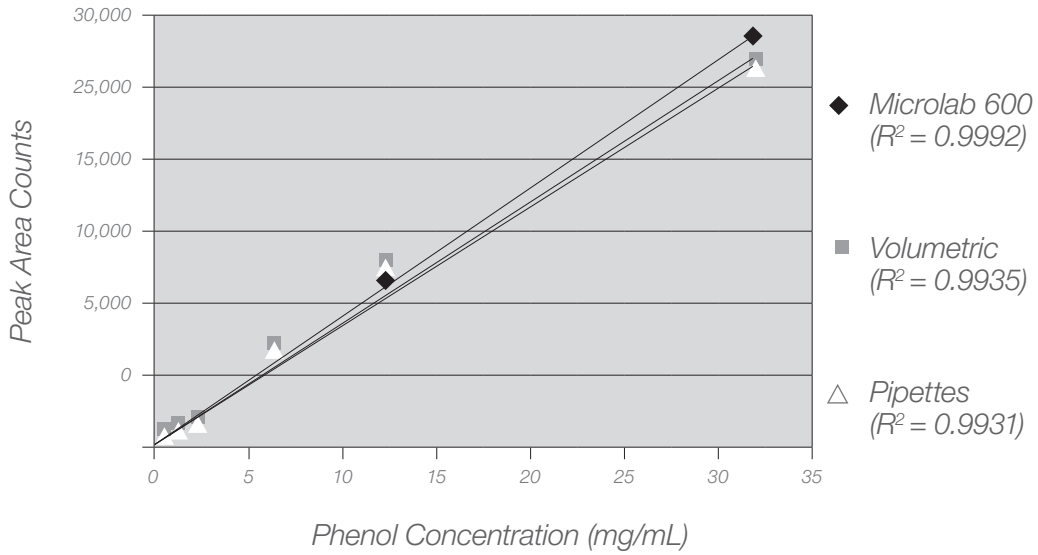

Figure 1: *Average Peak Area Counts from Dilutions Prepared Using the Microlab 600, Volumetric Glassware and Air Displacement Pipettes, Samples Analyzed via HPLC (n = 5).*

Data Analysis

The data below was generated from estimated labor and waste removal costs. The cost of acetronitrile was determined from the manufacturer (Sigma, 34851-4L). All other values in the table are based on data collected in this experiment. Table 3 was used to generate the data for the Pay Back Period below.

Table 3: Comparison of Cost, Time and Waste Generated for Each Technique

Parameter	Microlab 600	Volumetric Glassware	Pipettes
Acetronitrile Used (mL)	5.17	51.17	5.17
Acetronitrile Cost (0.078/mL)	$0.40	$3.99	$0.40
Average time (min)	2.95	13.15	5.38
Labor ($15/hour)	$0.74	$3.29	$1.35
Waste Generated (mL)	6	60	6
Waste Disposal Cost ($0.0008/mL)	$0.005	$0.05	$0.005
Total Cost Per Series	$1.15	$7.33	$1.76

Note: Dollar amounts are in USD.

Pay Back Period Calculation

The calculations below are making an assumption that five sample series are run per day and these are prepared and run five days a week.

Microlab 600 Comparison to Volumetric Glassware

Cost Per Series Savings $7.33 - $1.15 = $6.18

$6.18 x 5 (sample series) x 5 (days) = $154.50/week

$5500 / $154.50 = 36 weeks

Microlab 600 Comparison to Pipettes

Cost Per Series Savings $1.76 - $1.15 = $0.61

$0.61 x 5 (sample series) x 5 (days) = $15.25/week

$5500 / $15.25 = 361 weeks

Conclusion

When the Microlab 600 is compared directly with volumetric glassware there is a substantial savings of time, buffer and waste disposal. From the data collected in Table 3 we see that it is roughly four times faster to use the Microlab 600 than volumetric glassware and uses 10 times less reagent to prepare a sample series. The reason for these differences is simple; volumetric glassware requires more time because the glassware has to be cleaned between uses. Volumetric glassware vessel size is limiting, so the user is required to make more sample volume than would actually be consumed during the HPLC analysis. This wastes reagents and is ultimately not cost effective. When the Microlab 600 is compared to volumetric glassware there is a Pay Back Period of 36 weeks. It will take less than a year to collect on the investment of the Microlab 600.

When the Microlab 600 is compared to the air displacement pipettes there is a different result. The Microlab 600 and pipettes are essentially the same cost per sample series. There is not a substantial cost savings when using the Microlab 600 compared to the pipettes. However, the advantages of the Microlab 600 make this instrument a better choice. Air displacement pipettes are affected by atmospheric pressure and sample viscosity. In contrast, the Microlab 600 is a positive displacement pump that functions independently of solution viscosity or atmospheric pressure. As stated previously the different techniques are used in a wide range of industries where multiple users employ these technologies to prepare samples. Pipettes are known to have high user-to-user variability and thus may not be the best instrument to use. Additionally, the pipettes require the use of tips which adds more cost to sample preparation and also additional waste for tip disposal.

In conclusion, all techniques described in this study are highly accurate and precise. For the volumetric glassware there is a time hurdle and a cost issue. The pipettes lack versatility under different conditions such as atmospheric pressure or sample viscosity. The Microlab 600 addresses both of these problems. Figure 1 shows that the Microlab 600 has the best R^2 value when compared with volumetric glassware and air displacement pipettes. However, the R^2 values for the volumetric glassware and air displacement pipettes are still impressive. From a time and cost analysis, the Microlab 600 outperforms the volumetric glassware, and from a versatility standpoint, it outperforms air displacement pipettes.

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